PHYSICAL CHEMISTRY 2016

13th International Conference on
Fundamental and Applied Aspects of
Physical Chemistry

Proceedings
Volume I

B E L G R A D E
September 26 - 30, 2016

ISBN 978-86-82475-34-7
Title: Physical Chemistry 2016 (Proceedings)
Editors: Željko Čupić and Slobodan Anić
Published by: Society of Physical Chemists of Serbia, Studentski trg 12-16, 11158, Belgrade, Serbia.
Publisher: Society of Physical Chemists if Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: "Jovan", Printing and Publishing Company; 200 Copies.
Number of pages: 6+502; Format B5; printing finished in September 2016

Text and Layout: "Jovan"

200 - *Copy printing*

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13th International Conference on
Fundamental and Applied Aspects of
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Organized by

The Society of Physical Chemists of
Serbia

in co-operation with

Institute of Catalysis Bulgarian Academy of Sciences

and

Boreskov Institute of Catalysis Siberian Branch of
Russian Academy of Sciences

and

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THE EFFECTS OF FLUOXETINE ON HIPPOCAMPAL ANTIOXIDATIVE DEFENSE IN DEPRESSIVE-LIKE RATS

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ABSTRACT

Exposure of an organism to chronic social isolation (CSIS) causes modulation of antioxidant defense system in the brain which has been shown to have important role in depression. Fluoxetine (Flx) is the first-line treatment for depression; however, precise mechanism of its action still remains elusive. The aim of this study was to investigate the effect of 3 weeks of Flx treatment on malondialdehyde (MDA) level, an oxidative stress parameter as well as on the activities of GSH-dependent antioxidative enzymes in the hippocampus of rats exposed to 6 weeks of CSIS. Increased MDA content following CSIS and Flx treatment (controls or CSIS) of rats, suggests on hippocampal oxidative damage. CSIS induced reduction of hippocampal glutathione-S-transferase that was reversed by Flx treatment, as well as an increase in glutathione peroxidase/reductase activities. The present study contributes to our understanding of the mechanisms that underlie the antidepressant activity of Flx in rats exposed to CSIS, an animal model of depression.

INTRODUCTION

Psychosocial stress is one of the major factors that could contribute to depression. Chronic social isolation (CSIS), an animal model of depression, causes changes in redox state in the hippocampus which may be responsible for depressive-like behavior in rats [1]. Fluoxetine (Flx, Prozac) belongs to the class of the new antidepressive drugs and it has been used as a first line treatment for depression and anxiety [2]. It acts as a selective serotonin reuptake inhibitor but its precise mechanism of action still remains unknown. The aim of this study was to examine the effect of 3 weeks of Flx treatment (15 mg/kg/day) on malondialdehyde (MDA) level, an oxidative stress parameter, and glutathione (GSH)-related antioxidative defense, as indirect marker of oxidative stress, in the hippocampus of rats exposed to six weeks of CSIS. Hence, we measured the

glutathione (GSH) content, as the main redox buffer in the cell, and activities of GSH-dependent antioxidative enzymes including glutathione peroxidase (GPx), glutathione reductase (GLR) and glutathione-S-transferase (GST).

EXPERIMENTAL

Twenty four adult male Wistar rats, 2.5 months old, at the onset of the experiment served as subjects. Rats were housed under standard conditions (temperature-controlled environment (21-23°C), 12/12h light/dark cycle with food and water available *ad libitum*). Prior to stress exposure, the animals were housed in groups of four per cage and randomly divided into two groups. Control group consisted of four animals per cage while rats subjected to CSIS stress were housed individually for 6 weeks during which animals had normal auditory and olfactory experiences, but without any visual and tactile contact with other animals. Flx-HCl solution was administered daily by intraperitoneal (i.p.) injections of 15 mg/kg, during the last 3 weeks in both control (Con+Flx) and socially isolated (CSIS+Flx) rats. We reported in previous study that the chosen dose of Flx is the one corresponding to serum levels within the therapeutically effective dose range for the treatment in depressive patients [3]. Vehicle-treated animals received i.p. physiological saline injections (0.9 % NaCl) (Con+Veh and CSIS+Veh). Hippocampal cytosolic fractions were used for biochemical parameters determination. Protein concentrations were measured by the method of Lowry [4], using bovine serum albumin as a standard. GSH content was measured according to the method of Ellman [5]. We used spectrophotometric assays for measuring the activity of GPx, GLR and GST enzymes [6, 7, 8]. All data are displayed as mean ± S.E.M. and analyzed by two-way ANOVA followed by Duncan's post-hoc test.

RESULTS AND DISCUSSION

Significant increase in MDA content in CSIS+Veh group shows the signs of oxidative stress ($^{**}p<0.01$) (Figure 1). Interestingly, Flx treatment caused the increase in MDA content in both control and isolated ($^{**}p<0.01$) animals indicating its detrimental effect of lipid oxidation.

According to data presented in Table 1., CSIS led to slight decrease of GSH content that was not statistically significant ($p>0.05$). However, treatment with Flx induced increase of GSH level in Flx-treated CSIS animals ($^{\wedge}p<0.05$) as compared to vehicle-treated CSIS.

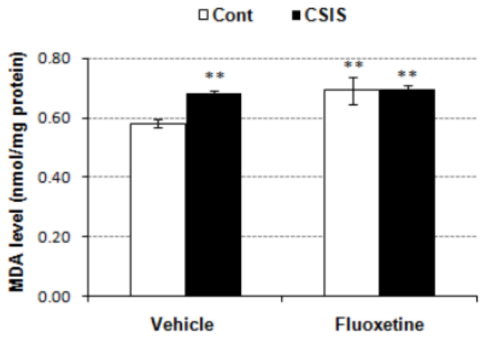

Figure 1. Changes of MDA content (nmol/mg protein) in hippocampus of control and chronic social isolation (CSIS) rats treated either with vehicle (0.9% NaCl), or fluoxetine. Asterisk indicates significant differences between all treated experimental groups and Con+Veh ($^{**}p<0.01$).

CSIS stress brought to distinguishable changes in GSH-dependent antioxidant enzyme activities. GPx activity was significantly increased in all three groups compared to Con+Veh, with the most significant changes observed in Con+Flx and CSIS+Flx ($^{***}p<0.001$), demonstrating the role of Flx in enhancing antioxidative defense. GLR activity didn't undergo any significant change in CSIS rats ($p>0.05$) but Flx treatment increased its activity in control ($^{*}p<0.05$) and CSIS ($^{**}p<0.01$) groups. Therefore, based on aforementioned results, Flx intensifies GSH-dependent antioxidative defense by stimulating the catalytic activity of both GPx and GLR.

Table1. Hippocampal GSH content and activity of GPx, GLR and GST enzymes of control and chronic social isolation (CSIS) rats treated either with vehicle (0.9% NaCl) or fluoxetine. Symbols indicate significant differences between: all treated experimental groups and Con+Veh, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; CSIS+Flx and CSIS+Veh $^{\wedge}p<0.05$, $^{\wedge\wedge\wedge}p<0.001$; CSIS+Flx and Con+Flx $^{\#}p<0.05$.

Groups	GSH content (nmol/mg protein)	GPx activity (mU/mg protein)	GLR activity (mU/mg protein)	GST activity (mU/mg protein)
Con + Vehicle	43.35 ± 2.32	25.10 ± 0.66	30.44 ± 0.68	122.55 ± 4.09
Con + Flx	47.70 ± 2.71	$31.22 \pm 0.77^{***}$	$33.31 \pm 0.93^{*}$	122.45 ± 5.17
CSIS + Vehicle	39.79 ± 1.23	$27.58 \pm 0.11^{**}$	31.26 ± 1.03	$99.72 \pm 2.76^{***}$
CSIS + Flx	$47.57 \pm 1.91^{\wedge}$	$29.35 \pm 0.61^{***\wedge\#}$	$33.91 \pm 0.35^{**\wedge}$	$123.16 \pm 2.08^{\wedge\wedge\wedge}$

The compromised GST activity in CSIS group ($^{***}p<0.001$) could be one of the contributing factors that led to the reduced consumption of GSH, a cosubstrate essential for its activity. Treatment of Flx restored GSTactivity in CSIS rats as compared to CSIS alone ($^{\wedge\wedge\wedge}p<0.001$) indicating that it interferes with CSIS-induces pathways of oxidative defense as well as its role in improvement of the cell capabilities in detoxification of drug. Moreover, increased GPx activity in vehicle-treated CSIS group and consequently consumption of GSH did not affect its level although increased GPx activity was not accompanied with increased activity of GLR enzyme responsible for GSH restoration, suggesting on de novo synthesis of GSH.

CONCLUSION

The data revealed that Flx may cause state of oxidative stress, judging by the overall MDA content increment in control rats. In addition, it unambiguously triggered the cellular defense against ROS by enhancing the activities of GPx, GLR as well as reversing GST activity to the normal level, thereby improving the cell capacity in toxic electrophile molecules removal, but relying on the fact that some GSTs may act as peroxidases.

Acknowledgement

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grants no. 173044).

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CIP - Каталогизација у публикацији - Народна библиотека Србије, Београд

544(082)
66.017/.018(082)
502/504(082)
663/664:658.56(082)
615.31:547(082)

INTERNATIONAL Conference on Fundamental and Applied Aspects of Physical Chemistry (13 ; 2016 ; Beograd)
Physical Chemistry 2016 : proceedings. Vol. 2 / 13th International Conference on Fundamental and Applied Aspects of Physical Chemistry, Belgrade, 26-30 September 2016 ; [editors Željko Čupić and Slobodan Anić]. - Belgrade : Society of Physical Chemists of Serbia, 2016 (Belgrade : Jovan). - IV, 507-930 str. : ilustr. ; 24 cm

Tiraž 200. - Bibliografija uz svaki rad.

ISBN 978-86-82475-33-0

1. Society of Physical Chemists of Serbia (Beograd)
a) Физичка хемија - Зборници b) Наука о материјалима - Зборници c) Животна средина - Заштита - Зборници d) Животне намирнице - Контрола квалитета - Зборници e) Фармацеутска хемија - Зборници
COBISS.SR-ID 225802508

08. 09. 2016